UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Environmental Carcinogenesis Division
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

DATE: 5 May 2000

SUBJECT: Report of the peer review of the thyroid histopathology from rodents and rabbits exposed to ammonium perchlorate in the drinking water.

FROM: Douglas C. Wolf, D.V.M., Ph.D. signature
ECD/NHEERL

TO: Annie Jarabek, M.S., NCEA
William Farland, Ph.D., Director, NCEA

A draft risk assessment entitled: Perchlorate Environmental Contamination: Toxicological review and risk characterization based on emerging information (NCEA-1-0503) underwent external peer review in February 1999. During the process of peer review of this document it was noted that the thyroid histopathology, which made a significant contribution to the risk assessment, had never been reviewed by a second pathologist in any of the studies. In addition, the animal studies had been performed at several different laboratories with several different study pathologists using different lesion grading schemes. This potential inconsistency between study reports made it difficult to directly compare one study to another and use the data consistently across studies for the development of a dose-response estimate. In response, the National Center for Environmental Assessment (NCEA) made the decision to have a single pathologist review all the studies using one consistent lesion grading scheme and convene a pathology working group (PWG).

NCEA requested that I perform this review because I had not been involved with any of the previous work performed with ammonium perchlorate. In addition, I had developed a thyroid grading scheme to analyze a similar thyroid response in rodents exposed to sodium chlorate that could be used to review these studies (Hooth MJ, DeAngelo AB, George MH, Boorman GA, Wolf DC: Sodium chlorate treatment results in a dose-dependent increase in rat thyroid follicular cell hyperplasia following subchronic exposure in drinking water. Toxicol Sci 54 (suppl): Abstr 1025, 218, 2000; Hooth MJ, Gaillard ET, George M, DeAngelo AB, Wolf DC: Sodium chlorate induced thyroid follicular cell hyperplasia in rats but not mice. Toxicol Pathol (manuscript in preparation).

Over the course of several months I received rodent and rabbit thyroid slides for examination along with the associated study specific paperwork from the Department of Defense and its contracted laboratories. I read each study according to the criteria which are detailed in the Experimental Pathology Laboratory, Inc. (EPL) PWG report. Each study was read separately and the slides

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were reviewed without prior knowledge to which treatment group they belonged. In my initial review of the paperwork associated with each study, it was apparent to me that the terminology used by each of the original study pathologists was not consistent across studies. This is not to say they were incorrect nor the diagnoses were inappropriate. Rather, that there could be confusion on the part of someone comparing the data across studies who may not have a thorough background in histopathology terminology or thyroid physiology and pathology. Therefore, an objective of the current review was to use the same criteria to assess the potential effect of ammonium perchlorate in all studies.

Only thyroid gland slides from the studies evaluated in the December 1998 external review draft EPA assessment Perchlorate Environmental Contamination: Toxicological review and risk characterization based on emerging information (NCEA-1-0503) were reviewed. These studies are as follows:

Argus Research Laboratories, Inc. (1998c). Oral (drinking water) developmental toxicity study of ammonium perchlorate in rabbits [report amendment: September 10]. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-002.

Keil, D.; Warren, A.; Bullard-Dillard, R.; Jenny, M.; EuDaly, J. (1998). Effects of ammonium perchlorate on immunological, hematological, and thyroid parameters. Charleston, SC: Medical University of South Carolina, Department of Medical Laboratory Sciences; report no. DSWA01-97-1-008.

Argus Research Laboratories, Inc. (1998a). A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [report amendment: July 27]. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1613-002.

Springborn Laboratories, Inc. (1998). A 90-day drinking water toxicity study in rats with ammonium perchlorate: amended final report [amended study completion date: June 3]. Spencerville, OH: Springborn Laboratories, Inc.; study no. 3455.1.

14-day oral dosing toxicity study of ammonium perchlorate administered in the drinking water to female Sprague-Dawley rats. WPAFB #A10 (Caldwell et al., 1995)

Argus Research Laboratories, Inc. (1998b). Oral (drinking water) two-generation (one litter per generation) reproduction study of ammonium perchlorate in rats. Horsham, PA: Argus Research Laboratories, Inc.; protocol no. 1416-001.

After my initial peer review of 100% of the slides presented to me. Peter Mann, D.V.M, an experienced quality assurance pathologist on contract from EPL, reviewed 100% of the slides for consistency of my read. Subsequent to Dr. Mann's review a PWG of 5 experienced veterinary pathologists reviewed a subset of the slides. For the results of the PWG please see: The effects of ammonium perchlorate on thyroids, Pathology working group report.

Several action items were identified by the PWG. These were for me to (1) re-examine the PND 5 pups from the multi-generation studies for colloid depletion as the PWG felt that too many of the control pups were diagnosed with colloid depletion due to my reading the study without knowledge of controls; (2) re-examine all thyroids for which there was a diagnosis of hyperplasia for the presence of hypertrophy in order to better assess the relationship between hypertrophy and hyperplasia; (3) re-examine all thyroids for which there was a diagnosis of hyperplasia using the

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PWG's criteria for hyperplasia.

I re-examined 100% of the PND5 pups for colloid depletion after reviewing the spectrum of colloid present in the control. All thyroids for which I had a diagnosis of hyperplasia were also examined for hypertrophy and re-examined for hyperplasia using slightly different criteria.

The attached tables reflect the summary of my final review subsequent to the PWG. The criteria used were:

Colloid depletion

Colloid depletion was considered present based on reduction or absence of colloid as evidenced by lack of eosinophilic protein in the follicular lumen or pale, lacy and/or granular material in the follicular lumen. Loss of colloid is considered a more sensitive indication of response to treatment-induced TSH increases than hypertrophy or hyperplasia. The data in the tables are presented as incidence of this occurrence.

Follicular cell hypertrophy

Follicular cell hypertrophy was considered present when thyroid follicles were uniformly lined by tall cuboidal to columnar epithelium. The cytoplasm was typically more basophilic than nonhypertrophic cells and had a lacy, sometimes vacuolated, appearance. There was an increase in cytoplasm to nuclear ratio along with an increased cell width and height. The data in the tables are presented as incidence of this occurrence.

Hyperplasia

Hyperplasia was graded as:

0 - follicles lined by normal appearing, squamous to short cuboidal epithelium with eosinophilic cytoplasm and normochromic nuclei.

1- scattered individual or sometimes two adjacent follicles that have focal hyperplasia within the follicle. The areas of focal hyperplasia within a follicle were characterized by multiple layers of follicular epithelium usually 2-3 cells thick protruding into the lumen of the follicle. There had to be 2 or more hyperplastic follicles and follicles on the peripheral rim of the thyroid gland section were not counted.

2 - a greater number of scattered individual affected follicles or foci of more than 2 hyperplastic follicles. The areas of focal hyperplasia within a follicle were characterized by multiple layers of cuboidal follicular epithelium, usually more than 3 layers, protruding into the follicular lumen. These areas of hyperplasia could also have microfollicular formation within them.

Hyperplasia is reported in the tables as incidence of this occurrence and as the mean severity of the hyperplasia of those animals with hyperplasia. Animals with a hyperplasia grade of 0 were not included in the group mean severity score presented in the tables.

Adenoma

Adenoma was diagnosed according to standard criteria used by the National Toxicology Program

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which are detailed in the previously cited PWG report. Adenoma were reported as incidence of this occurrence.

Discussion

The attached summary tables do not reflect statistical significance as that analysis was not performed as part of this review. The consensus opinion of the PWG and Dr. Wolf was that there is a morphologically evident response to ammonium perchlorate treatment in the thyroids of rabbits, rats and mice. The thyroid response is also present in neonatal animals. Morphological alterations present and associated with ammonium perchlorate treatment are follicular colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia. There appears to be an association between hypertrophy and hyperplasia in that hyperplasia did not occur without coincident hypertrophy. This may suggest that these two responses are not due to different pathways but are different manifestations of the proposed mechanism of ammonium perchlorate-induced thyroid alterations. No observed effect levels were not determined as part of this review.

The evaluation of the 14-day oral dosing toxicity study of ammonium perchlorate administered in the drinking water to female Sprague-Dawley rats. WPAFB #A10 (Caldwell et al., 1995) was very difficult because of the non-routine staining method. It appears that PAS reaction with a green counterstain was used. The rest of the studies were apparently stained using routine hematoxylin and eosin.

In addition to the above lesions, two animals from the same dose group in one study had adenomas and one of these animals had two adenomas for a total of 3 adenomas in 2 animals. These adenomas are thought to be treatment related. The background incidence of thyroid follicular cell adenomas in male F344 rats after 2 years on study is 38/3419 rats from 67 NTP studies or 1.1% incidence at 2 years. In the current series of studies 2/30, or 6.7% of the group, developed thyroid follicular cell adenomas after treatment with 30 mg/kg/day ammonium perchlorate in the drinking water from conception to 19 weeks of age (adult male F1).

In summary, ammonium perchlorate when given in the drinking water causes thyroid follicular colloid depletion, follicular cell hypertrophy and hyperplasia in multiple species. There is evidence that ammonium perchlorate may cause thyroid follicular adenomas in male rats.

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Table 1. 14-day oral dosing toxicity study of ammonium perchlorate administered in the drinking water to female Sprague-Dawley rats. WPAFB #A10 (Caldwell et al., 1995). (EPL Study #'s 480-030, 416-002-8)

Dose Group	Dose of Perchlorate (mg/L)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	6	3	0	0	
2	1.25	6	1	0	0	
3	5	6	2	1	0	
4	12.5	6	1	0	0	
5	25	6	6	4	2	1.5 +/- 0.7
6	50	6	6	5	1	1
7	125	6	6	6	3	1
8	250	6	6	6	1	2

Table 2. 14-day oral dosing toxicity study of ammonium perchlorate administered in the drinking water to male Sprague-Dawley rats. WPAFB #A10 (Caldwell et al., 1995). (EPL Study #'s 480-030, 416-002-8)

Dose Group	Dose of Perchlorate (mg/L)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	6	3	4	2	1
2	1.25	6	5	5	2	1
3	5	6	6	4	2	1
4	12.5	6	6	5	3	1
5	25	6	5	1	0	
6	50	6	6	5	0	
7	125	6	6	6	5	1.2 +/- 0.5
8	250	6	6	6	4	1

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Table 3. Subchronic, 90-day, drinking water ammonium perchlorate toxicity study in female rats with a 30-day recovery. Interim necropsy, 14 days of treatment. Springborn Labs, SLI3455.1. (EPL Study #'s 480-029, 416-002-7)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	0	1	0	
2	0.01	10	0	1	0	
3	0.05	10	0	0	0	
4	0.2	10	0	0	0	
5	1.0	10	0	1	0	
6	10.0	10	4	8	0	

Table 4. Subchronic, 90-day, drinking water ammonium perchlorate toxicity study in female rats with a 30-day recovery. Interim necropsy, 90 days of treatment. Springborn Labs, SLI3455.1. (EPL Study #'s 480-029, 416-002-7)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	0	0	0	
2	0.01	10	0	0	0	
3	0.05	10	1	3	0	
4	0.2	10	1	2	0	
5	1.0	10	1	1	0	
6	10.0	10	4	5	3	1

Table 5. Subchronic, 90-day, drinking water ammonium perchlorate toxicity study in female rats with a 30-day recovery. Terminal necropsy, 90 days of treatment, 30 days of recovery. Springborn Labs, SLI3455.1. (EPL Study #'s 480-029, 416-002-7)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	0	0	0	
3	0.05	10	0	0	0	
5	1.0	10	0	1	0	
6	10.0	10	0	0	0	

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Table 6. Subchronic, 90-day, drinking water ammonium perchlorate toxicity study in male rats with a 30-day recovery. Interim necropsy, 14 days of treatment. Springborn Labs, SLI3455.1. (EPL Study #'s 480-029, 416-002-7)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	7	3	4	3	1
2	0.01	10	0	5	1	1
3	0.05	10	5	5	2	1
4	0.2	10	5	3	0	
5	1.0	10	4	7	4	1
6	10.0	9	7	5	2	1

Table 7. Subchronic, 90-day, drinking water ammonium perchlorate toxicity study in male rats with a 30-day recovery. Interim necropsy, 90 days of treatment. Springborn Labs, SLI3455.1. (EPL Study #'s 480-029, 416-002-7)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	0	1	0	
2	0.01	10	1	2	0	
3	0.05	10	1	0	0	
4	0.2	10	1	2	0	
5	1.0	10	2	3	0	
6	10.0	10	9	8	4	1

Table 8. Subchronic, 90-day, drinking water ammonium perchlorate toxicity study in male rats with a 30-day recovery. Terminal necropsy, 90 days of treatment, 30 days of recovery. Springborn Labs, SLI3455.1. (EPL Study #'s 480-029, 416-002-7)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	1	2	1	1
3	0.05	10	3	4	3	1
5	1.0	10	0	2	1	1
6	10.0	10	3	0	0	

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Table 9. Neurobehavioral development study of ammonium perchlorate administered in drinking water to F0 generation female rats. 1613-002. (EPL Study #'s 480-028, 416-002-6)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	25	5	12	4	1
2	0.1	25	12	13	1	1
3	1	25	20	18	6	1
4	3	25	11	11	3	1
5	10	25	11	12	7	1

Table 10. Neurobehavioral development study of ammonium perchlorate administered in drinking water to rats. PND5 female rats. Argus Lab #1613-002. (EPL Study #'s 480-031, 416-002-2)

Dose Group	Dose of Perchlorate (mg/L)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	2	0	0	0
2	0.1	10	7	0	0	1
3	1	10	1	1	1	1
4	3	10	7	6	2	1.2
5	10	10	8	3	1	1

Table 11. Neurobehavioral development study of ammonium perchlorate administered in drinking water to rats. PND5 male rats. Argus Lab #1613-002. (EPL Study #'s 480-031, 416-002-2)

Dose Group	Dose of Perchlorate (mg/L)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	10	4	0	0	1
2	0.1	10	2	0	0	1
3	1	10	7	0	0	1
4	3	10	7	2	0	1
5	10	10	10	6	0	1

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Table 12. Neurobehavioral development study of ammonium perchlorate administered in drinking water to rats. Adult female rats. Argus Lab #1613-002, study 25F. (EPL Study #'s 480-031, 416-002-3).

Dose Group	Dose of Perchlorate (mg/L)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
5	0	10	5	0	1	1
4	0.1	10	4	0	0	1
3	1	10	8	2	0	1
2	3	10	10	2	0	1
1	10	10	8	2	1	1

Table 13. Neurobehavioral development study of ammonium perchlorate administered in drinking water to rats. Adult male rats. Argus Lab #1613-002, study 25F. (EPL Study #'s 480-031, 416-002-3).

Dose Group	Dose of Perchlorate (mg/L)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
5	0	10	6	1	1	1
4	0.1	10	2	1	0	
3	1	10	2	1	0	
2	3	10	3	1	0	
1	10	10	8	1	0	

Table 14. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats, first parental generation (P1) female rats. Argus #1416-001. (EPL Study #'s 480-032, 416-002-9)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	27	14	2	0	
2	0.3	21	11	3	0	
3	3	26	21	13	0	
4	30	24	24	17	6	1

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Table 15. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats, first parental generation (P1) male rats. Argus #1416-001. (EPL Study #'s 480-032, 416-002-9

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	30	25	16	2	1
2	0.3	30	22	12	1	1.1
3	3	30	24	22	5	1.2 +/- 0.5
4	30	30	30	24	8	1

Table 16. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats: F1 generation, second parental generation (P2) female rats. Argus #1416-001 (EPL Study #'s 480-033, 416-002-10)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	20	7	2	1	1
2	0.3	27	1	4	1	1
3	3	27	0	5	3	1
4	30	23	21	12	0	

Table 17. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats: F1 generation, second parental generation (P2) male rats. Argus #1416-001 (EPL Study #'s 480-033, 416-002-10)

Dose Group	Dose of Perchlorate mg/kg/day	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia	Adenoma
1	0	29	3	8	5	1	
2	0.3	30	4	8	5	1	
3	3	30	16	16	11	1	
4	30	30	27	27	13	1.1 +/- 0.3	2

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Table 18. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats: F1 and F2 weanlings. first weanling generation (F1) female rats. Argus #1416-001 (EPL Study #'s 480-034, 416-002-11)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	28	2	3	0	
2	0.3	22	3	7	1	1
3	3	24	5	15	1	1
4	30	21	21	21	7	1

Table 19. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats: F1 and F2 weanlings. first weanling generation (F1) male rats. Argus #1416-001 (EPL Study #'s 480-034, 416-002-11)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	28	1	2	0	
2	0.3	22	3	4	0	
3	3	25	4	14	5	1
4	30	23	18	23	6	1

Table 20. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats: F1 and F2 weanlings. second weanling generation (F2) female rats. Argus #1416-001 (EPL Study #'s 480-034, 416-002-11)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	20	0	0	0	0
2	0.3	27	3	1	0	1
3	3	28	20	8	2	1
4	30	25	23	13	1	1

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Table 21. Oral (drinking water) 2-generation (1 litter per generation) reproduction study of ammonium perchlorate in rats: F1 and F2 weanlings. second weanling generation (F2) male rats. Argus #1416-001 (EPL Study #'s 480-034, 416-002-11)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	20	0	0	0	
2	0.3	27	2/27 (7)	0	0	
3	3	28	20/28 (71)	4	0	
4	30	25	19/25 (76)	7	0	

Table 22. Oral (drinking water) developmental toxicity study of ammonium perchlorate in rabbits, effects in thyroids of maternal rabbits. Argus ID#1416-002. (EPL Study #'s 480-025, 416-002-1)

Dose Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
1	0	25	0	5	0	
2	0.1	25	1	3	0	
3	1	25	4	1	0	
4	10	25	14	18	10	1
5	30	25	15	18	15	1.3 +/- 0.5
6	100	25	14	20	16	1.2 +/- 0.4

Table 23. Effects of ammonium perchlorate in thyroid gland of male B6C3F1 mice after 90 days of treatment. Medical University of SC. (EPL Study #'s 480-027; 416-002-5)

Study Group	Dose of Perchlorate (mg/kg/day)	Number of Animals	Decreased Colloid	Follicular Cell Hypertrophy	Follicular Cell Hyperplasia	Mean Severity of Hyperplasia
A	0	6	0	0	0	0
A	0.1	6	0	1	0	0
A	1	6	0	1	0	0
A	3	6	0	2	0	0
A	30	6	2	4	3	2
D	0	6	0	0	0	0
D	0.1	6	0	0	0	0
D	1	6	0	0	0	0
D	3	6	0	0	0	0
D	30	5	1	4	1	1

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